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In-silico determination of *Pichia pastoris* signal peptides for extracellular recombinant protein production

Aslan Massahi [a,b], Pınar Çalık [a,b,*]

[a] Industrial Biotechnology and Metabolic Engineering Laboratory, Chemical Engineering Department, Middle East Technical University, 06800 Ankara, Turkey
[b] Department of Biotechnology, Graduate School of Natural and Applied Sciences, Middle East Technical University, 06800 Ankara, Turkey

HIGHLIGHTS

- Promising endogenous secretory signal peptides of *Pichia pastoris* were identified.
- The highest endogenous *D*-scores obtained were: 0.932, 0.918, and 0.910.
- Eight signal peptides had *D*-score higher than that of *Saccharomyces cerevisiae* α-mating factor.
- Verified secretory signal peptides had signal peptide-ness score (*D*-score) of >0.8.
- Overall, SignalP, Phobius, and WolfPsort predicted 82% same cleavage sites.

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ABSTRACT

In-silico identified novel secretory signal peptides (SPs) are required in vivo to achieve efficient transfer or to prevent other cellular proteins from interfering with the process in extracellular recombinant protein (r-protein) production. 56 endogenous and exogenous secretory SPs, have been used or having the potential to be used in *Pichia pastoris* for r-protein secretion, were analyzed in-silico using the softwares namely SignalP4.1, Phobius, WolfPsort0.2, ProP1.0, and NetNGlyc1.0. Among the predicted 41 endogenous secretory SPs, five of them have been used in *P. pastoris,* and regarded as positive controls; whereas, 36 of them have not been used. Amongst, the predicted cleavage site for each of the 32 endogenous secretory SPs was found to be same by the three programs. The secretory SPs having the highest *D*-scores, the score quantifying the signal peptide-ness of a given sequence segment, were: MKILSALLLLFTLAFA ($D=0.932$), MRPVLSLLLLASSVLA ($D=0.932$), MFKSLCMLIGSCLLSSVLA ($D=0.918$). As *D*-scores of these SPs are higher than that of *Saccharomyces cerevisiae* α-mating factor signal peptide which has been widely used for r-protein production, they can be considered as the promising candidates. Among the predicted 15 exogenous SPs, 11 have been used in *P. pastoris*: therefore, they were evaluated as positive controls. The three programs predicted a unique cleavage site for each of the 10 exogenous SPs; and *D*-scores of these SPs were within $D=0.805$–0.900; whereas, four exogenous secretory SPs, which have not been used in *P. pastoris*, have *D*-scores within $D=0.494$–0.702.

1. Introduction

Signal peptides (SPs) associate synthesized secretory proteins with membrane by sponsoring transfer of the attached protein into the membrane; thus, a novel signal peptide (SP) based on metabolic engineering design commences a new era in extracellular recombinant protein (r-protein) production. In recent years, the yeast *Pichia pastoris* has become one of the most successful and popular host systems for heterologous protein production, as it grows rapidly on an inexpensive minimal medium at high cell densities and secretes the r-protein(s) to the fermentation medium which, consequently, simplifies the downstream processes. For r-protein production, among the crucial steps which include several physiological and genetic factors, utilizing a secretory SP for transferring the r-protein to the extracellular medium is one of the challenging steps (Çelik and Çalık, 2012). In spite of its importance in extracellular protein production, the choice of a secretory SP is rather arbitrary and it is based on try and error experiments (Cereghino et al., 2002; Damasceno et al., 2012;

* Corresponding author at: Department of Chemical Engineering, Middle East Technical University, 06800 Ankara, Turkey. Tel.: +90 312 210 43 85; fax: +90 312 210 26 00.
E-mail address: pcalik@metu.edu.tr (P. Çalık).

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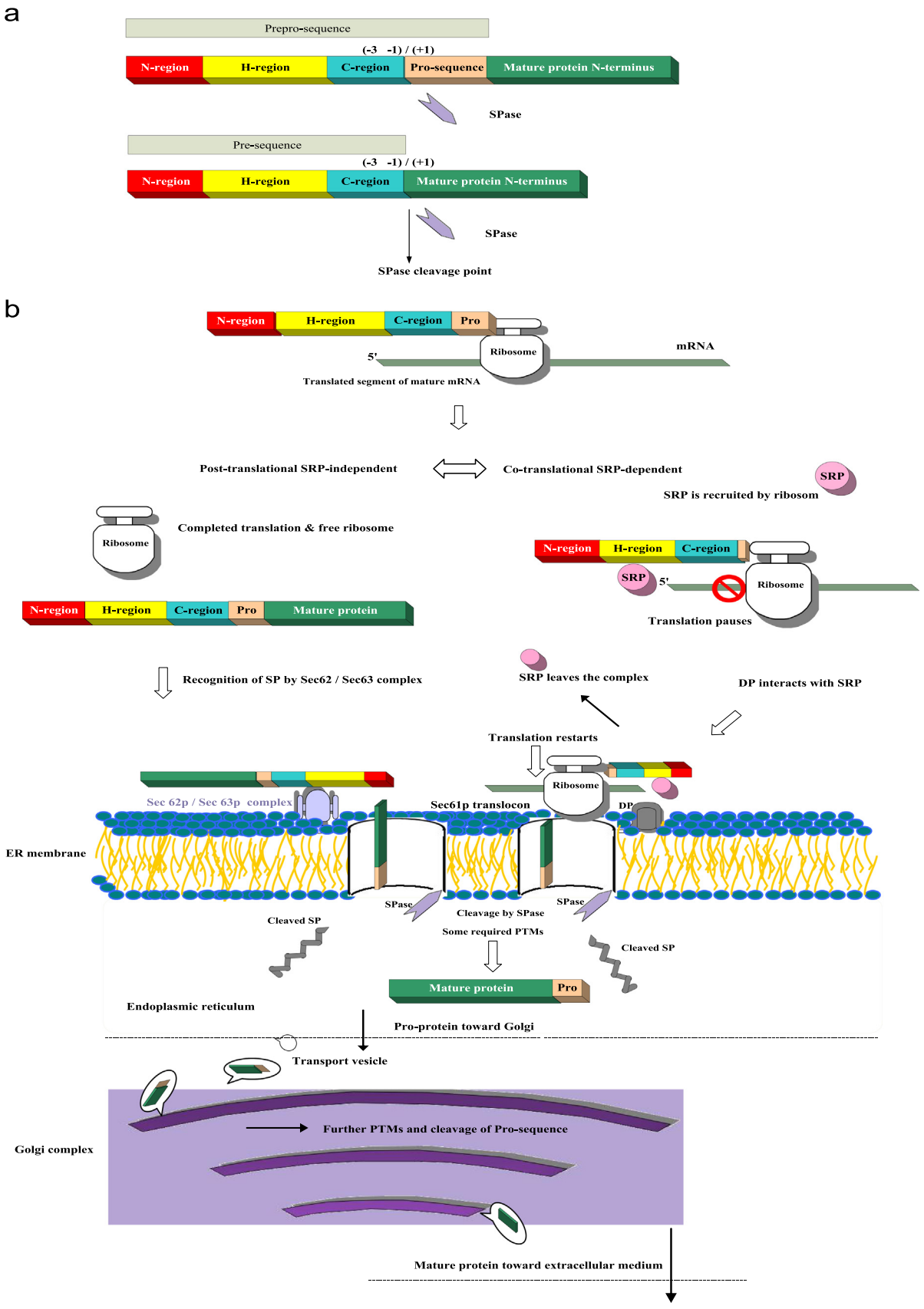

Fig. 1. Schematic representation of tripartite structure of a secretory SP (a), and two Sec-dependent translocation pathways in eukaryotes (b). SPase: signal peptidase I enzyme, SRP: signal recognition particle, DP: docking protein, Pro: pro-sequence, ER: endoplasmic reticulum, PTMs: Post-translational modifications.